

P-08-508/509 MR# 313164

Attachment 89 cont'd

[] Static, Acute,
96-Hour Limit Test with Rainbow Trout, *Oncorhynchus mykiss*

Revision 1
DuPont-[]

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study was conducted in compliance with U.S. EPA TSCA (40 CFR part 792) Good Laboratory Practice Standards, which are compatible with current OECD and MAFF (Japan) Good Laboratory Practice.

Study Director: [] — 14 July 2008
Date

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Attachment 89 cont'd

[] Static, Acute,
96-Hour Limit Test with Rainbow Trout, *Oncorhynchus mykiss*

Revision 1
DuPont-[]

QUALITY ASSURANCE STATEMENT

Work Request Number: []
Service Code Number: []

Key inspections for DuPont work request [], service code [] were performed for the tasks completed at DuPont by the Quality Assurance Unit of DuPont and the findings were submitted on the following dates.

<i>Phase Audited</i>	<i>Audit Dates</i>	<i>Date Reported to Study Director</i>	<i>Date Reported to Management</i>
Protocol:	May 30, 2007	May 30, 2007	May 30, 2007
Conduct:	June 4, 2007	June 4, 2007	June 4, 2007
Report/Records:	November 29-30, 2007	November 30, 2007	December 4, 2007
Report Revision 1:	July 8, 2008	July 8, 2008	July 8, 2008

Reported by: []

11 July 2008
Date

Attachment 89 cont'd

[] Static, Acute,
96-Hour Limit Test with Rainbow Trout, *Oncorhynchus mykiss*

Revision 1
DuPont-[]

CERTIFICATION

We, the undersigned, declare that this report provides an accurate evaluation of data obtained from this study.

Reviewed by:

[]

[]

9 July 2008
Date

Issued by Study Director:

[]

[]

14 July 2008
Date

TABLE OF CONTENTS

	Page
GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT	2
QUALITY ASSURANCE STATEMENT	3
CERTIFICATION	4
LIST OF TABLES	6
LIST OF APPENDICES	6
STUDY INFORMATION	7
REASON FOR REVISION 1.....	8
SUMMARY	8
INTRODUCTION.....	9
MATERIALS AND METHODS	9
A. Test Guidelines	9
B. Test Substance	9
C. Test Solution Preparation.....	9
D. Dilution Water	9
E. Test Organism Culture.....	9
F. Test Methods.....	10
G. Sample Preparation and Chemical Analysis	11
RESULTS AND DISCUSSION	12
A. In-Life Data.....	12
CONCLUSION	12
RECORDS AND SAMPLE STORAGE	12
REFERENCES.....	13
TABLES.....	14
APPENDICES	19

REASON FOR REVISION 1

Consistent reporting of endpoints across studies, based on guidance contained in OECD TG 201, 202 and 203.

SUMMARY

The acute toxicity of [] to unfed fingerling rainbow trout, *Oncorhynchus mykiss* was determined in an unaerated, static, acute, 96-hour limit test. The test was conducted in accordance with the OECD Guideline for the Testing of Chemicals: 203.

The study was conducted with a 120 mg/L nominal limit concentration of [] and a dilution water control at a mean temperature of 11.8°C (range of 11.7-12.4°C). The mean, measured [] limit test concentration was 96.9 mg/L. The mean, measured limit test concentration was 80-120% of the nominal limit test concentration for the study. A single dilution water control test chamber and 3 replicate limit test concentration chambers with 10 fish in each chamber were used for testing (total of 10 fish in the dilution water control and 30 fish in the limit test concentration). Fish in the dilution water control ranged from 4.3 to 4.9 cm in standard length (mean 4.47 cm), and 0.941 to 1.493 g in wet weight, blotted dry (mean 1.068 g) at test end. Control loading at test end was 0.534 g/L.

No mortality was seen at the nominal 120 mg/L (96.9 mg/L mean, measured) limit test concentration of [] or in the control at the end of the 96-hour limit test. The 96-hour LC₅₀, based on the nominal limit test concentration of [], was greater than 120 mg/L.

The results are summarized as follows:

Nominal concentration of [], mg/L ^a	dilution water control and 120
Mean, measured concentration of [], mg/L	ND ^b and 96.9
96-hour LC ₅₀ for [], based on nominal concentration, mg/L	greater than 120

a Not adjusted for [] purity by analysis during preparation.

b ND denotes none detected at or above the limit of detection of 0.0001µg/L.

RESULTS AND DISCUSSION

A. In-Life Data

1. Definitive Study

The nominal limit test concentration (not adjusted for [] purity during preparation) for the definitive study was 120 mg/L. A dilution water control was used in this study. No [] was detected in the dilution water control. The mean, measured concentration of [] was 96.9 mg/L and was within 80-120% of the nominal limit test concentration.

Dilution water quality was acceptable based on OECD⁽¹⁾ dilution water criteria. Based on the most recent semi-annual dilution water analysis (Table 1), contaminant concentrations were below concentrations that could be expected to affect the integrity of a study. All chemical and physical parameters for the definitive test (Tables 2 - 5) were within expected ranges. Total alkalinity, EDTA hardness, and conductivity of the dilution water control and limit test concentration at test start ranged from 51 to 53 mg/L as CaCO₃, 126 to 132 mg/L as CaCO₃, and 255 to 280 µmhos/cm, respectively. During the test, dissolved oxygen concentrations ranged from 7.4 to 9.7 mg/L, pH ranged from 7.2 to 7.7, and mean temperature was 11.8°C with a range of 11.7 to 12.4°C.

At test conclusion, fish from the dilution water control ranged from 4.3 to 4.9 cm in standard length (mean 4.47 cm) and 0.941 to 1.493 g in wet weight, blotted dry (mean 1.068 g). Standard length of the longest fish was not more than twice the length of the shortest fish in the control. Loading in the water control was 0.534 g/L at test conclusion.

Data on daily mortality and sublethal effects are presented in Tables 6 and 7, respectively. No mortality or sublethal effects were seen in the dilution water control or at the nominal 120 mg/L (96.9 mg/L mean, measured) [] concentration at the end of 96 hours. The 96-hour LC₅₀, based on the nominal [] limit test concentration and mortality, was greater than 120 mg/L.

CONCLUSION

[] was assessed for acute toxicity to unfed fingerling rainbow trout, *Oncorhynchus mykiss*, in an unaerated, static, acute, 96-hour limit test. The 96-hour LC₅₀, based on the nominal limit test concentrations of [] and mortality, was greater than 120 mg/L.

RECORDS AND SAMPLE STORAGE

Specimens (if applicable), raw data, the protocol, amendments (if any), and the final report will be retained at DuPont Haskell, Newark, Delaware, or at Iron Mountain Records Management, Wilmington, Delaware.

Attachment 89 cont'd

[] Static, Acute,
96-Hour Limit Test with Rainbow Trout, *Oncorhynchus mykiss*

Revision 1
DuPont-[]

Table 2
Water Chemistry of the Dilution Water Control and Limit Test Concentration at Test Start

Nominal [] Concentration (mg/L)	Total Alkalinity (mg/L as CaCO ₃)	EDTA Hardness (mg/L as CaCO ₃)	Conductivity (µmhos/cm)
Dilution Water Control	51	132	255
120	53	126	280

Table 3
Dissolved Oxygen Concentration (mg/L)^Ψ of [] Test Solutions

Nominal [] Concentration (mg/L)	0 Hours	24 Hours	48 Hours	72 Hours	96 Hours
Dilution Water Control	9.6	8.0	7.7	7.7	7.7
120 A†	9.7	8.2	7.7	8.0	8.0
120 B†	9.6	8.1	7.6	7.9	8.0
120 C†	9.7	7.9	7.4	7.7	7.7

Ψ The theoretical dissolved oxygen concentrations at 100% saturation is 9.1 mg/L at 20°C.

† Replicate test chambers contained 10 fish each at test start.

Attachment 89 cont'd

[] Static, Acute,
96-Hour Limit Test with Rainbow Trout, *Oncorhynchus mykiss*

Revision 1
DuPont-[]

Table 4
pH of [] Test Solutions

Nominal [] Concentration (mg/L)	0 Hours	24 Hours	48 Hours	72 Hours	96 Hours
Dilution Water Control	7.3	7.3	7.2	7.2	7.2
120 A†	7.7	7.5	7.4	7.3	7.2
120 B†	7.7	7.5	7.4	7.3	7.3
120 C†	7.7	7.5	7.3	7.2	7.2

† Replicate test chambers contained 10 fish each at test start.

Table 5
Temperature (°C) of [] Test Solutions

Nominal [] Concentration (mg/L)	0 Hours	24 Hours	48 Hours	72 Hours	96 Hours
Dilution Water Control	12.3	11.7	11.8	11.7	11.7
120 A†	12.3	11.7	11.8	11.7	11.7
120 B†	12.4	11.7	11.7	11.7	11.7
120 C†	12.4	11.7	11.7	11.7	11.7

† Replicate test chambers contained 10 fish each at test start.

Attachment 89 cont'd

[] Static, Acute,
96-Hour Limit Test with Rainbow Trout, *Oncorhynchus mykiss*

Revision 1
DuPont-[]

Table 6
Mortality of Rainbow Trout, *Oncorhynchus mykiss*, in an Unaerated Static, Acute, 96-Hour
Limit Test with []

Nominal [] Concentration (mg/L)	Number Dead / Number at Study Start			
	24 Hours	48 Hours	72 Hours	96 Hours
Dilution Water Control	0/10	0/10	0/10	0/10
120 A†	0/10	0/10	0/10	0/10
120 B†	0/10	0/10	0/10	0/10
120 C†	0/10	0/10	0/10	0/10

† Replicate test chambers contained 10 fish each at test start.

Table 7
Sublethal Effects of Rainbow Trout, *Oncorhynchus mykiss*, in an Unaerated Static, Acute,
96-Hour Limit Test with []

Nominal [] Concentration (mg/L)	Number with Effects / Number Alive			
	24 Hours	48 Hours	72 Hours	96 Hours
Dilution Water Control	0/10	0/10	0/10	0/10
120 A†	0/10	0/10	0/10	0/10
120 B†	0/10	0/10	0/10	0/10
120 C†	0/10	0/10	0/10	0/10

† Replicate test chambers contained 10 fish each at test start.

Attachment 90 cont'd

[] Static, Acute,
48-Hour Limit Test with *Daphnia magna*

Revision 1
DuPont-[]

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study was conducted in compliance with U.S. EPA TSCA (40 CFR part 792) Good Laboratory Practice Standards, which are compatible with current OECD and MAFF (Japan) Good Laboratory Practices.

Study Director:

[]

[]

14 July 2008
Date

Attachment 90 cont'd

[] Static, Acute,
48-Hour Limit Test with *Daphnia magna*

Revision 1
DuPont-[]

QUALITY ASSURANCE STATEMENT

Work Request Number: []
Service Code Number: []

Key inspections for DuPont work request [], service code [] were performed for the tasks completed at DuPont by the Quality Assurance Unit of DuPont and the findings were submitted on the following dates.

<i>Phase Audited</i>	<i>Audit Dates</i>	<i>Date Reported to Study Director</i>	<i>Date Reported to Management</i>
Protocol:	June 1, 2007	June 1, 2007	June 1, 2007
Conduct:	June 5, 2007	June 5, 2007	June 5, 2007
Report/Records:	November 30, 2007	December 3, 2007	December 4, 2007
Report Revision 1:	July 8, 2008	July 8, 2008	July 8, 2008

Reported by: []

[]
11 July 2008
Date

Attachment 90 cont'd

[] Static, Acute,
48-Hour Limit Test with *Daphnia magna*

Revision 1
DuPont-[]

CERTIFICATION

We, the undersigned, declare that this report provides an accurate evaluation of data obtained from this study.

Reviewed by:

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9 July 2008
Date

Issued by Study Director:

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[]

14 July 2008
Date

TABLE OF CONTENTS

	Page
GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT	2
QUALITY ASSURANCE STATEMENT	3
CERTIFICATION	4
LIST OF TABLES	6
LIST OF APPENDICES	6
STUDY INFORMATION	7
REASON FOR REVISION 1	8
SUMMARY	8
INTRODUCTION	9
MATERIALS AND METHODS	9
A. Test Guidelines	9
B. Test Substance	9
C. Test Solution Preparation	9
D. Dilution Water	9
E. Test Organism Culture	9
F. Test Methods	10
G. Sample Preparation and Chemical Analysis	10
RESULTS AND DISCUSSION	12
H. In-Life Data	12
CONCLUSION	12
RECORDS AND SAMPLE STORAGE	12
REFERENCES	12
TABLES	13
APPENDICES	19

REASON FOR REVISION 1

Consistent reporting of endpoints across studies, based on guidance contained in OECD TG 201, 202 and 203.

SUMMARY

The acute toxicity of [] to unfed *Daphnia magna* neonates, less than 24 hours old at test start, was determined in an unaerated, static, acute, 48-hour limit test. The test was conducted in accordance with the OECD Guideline for the Testing of Chemicals: 202.

The study was conducted with a 120 mg/L nominal concentration of [] and a dilution water control at a mean temperature of 20.4°C (range of 20.1-20.7°C). The nominal [] limit test concentration was 120 mg/L and the mean, measured concentration was 102 mg/L. The mean, measured limit test concentration was 80-120% of the nominal limit test concentration for the study. Six replicates with five daphnids per replicate were used for the limit test concentration and four replicates with five daphnids per replicate were used for the dilution water control.

No immobility was seen at the nominal 120 mg/L (102 mg/L mean, measured) [] limit test concentration at the end of the 48-hour limit test. No immobility or sublethal effects were seen in the dilution water control daphnids. The 48-hour EC₅₀, based on the nominal [] limit test concentration and immobility, was greater than 120 mg/L.

The results are summarized as follows:

Nominal concentration of [], mg/L ^a	dilution water control and 120
Mean, measured concentration of [], mg/L	ND ^b , and 102
48-hour EC ₅₀ for [], based on nominal concentration, mg/L	greater than 120

a Not adjusted for [] purity by analysis during preparation.

b ND denotes none detected at or above the limit of detection of 0.0001 µg/L.

RESULTS AND DISCUSSION

H. In-Life Data

1. Definitive Study

The nominal limit test concentration (not adjusted for [] purity during preparation) for the definitive study was 120 mg/L. A dilution water control was used in this study. Four replicate chambers were used for the dilution water control. Six chambers were used per limit test concentration with five daphnids in each chamber (20 daphnids per control and 30 per limit test concentration). The mean, measured concentration of [] was 102 mg/L and was within 80-120% of the nominal limit test concentration. No [] was detected in the dilution water control.

Dilution water quality was acceptable based on OECD⁽¹⁾ dilution water criteria. Based on the most recent semi-annual dilution water analysis (Table 1), contaminant concentrations were below concentrations that could be expected to affect the integrity of a study. All chemical and physical parameters for the definitive test (Tables 3 - 6) were within expected ranges. Total alkalinity, EDTA hardness, and conductivity of the dilution water control and limit test concentration at test start ranged from 51 to 52 mg/L as CaCO₃, 127 to 131 mg/L as CaCO₃, and 255 to 285 µmhos/cm, respectively. During the test, dissolved oxygen concentrations ranged from 8.5 to 8.8 mg/L, pH ranged from 7.4 to 8.0, and mean temperature was 20.4°C with a range of 20.1 to 20.7°C.

Data on daily immobility and sublethal effects are presented in Tables 7 and 8, respectively. No immobility or sublethal effects were seen in the dilution water or at the nominal 120 mg/L (102 mg/L mean, measured) [] limit test concentration at the end of 48 hours. The 48-hour EC₅₀, based on the nominal [] limit test concentration and immobility, was greater than 120 mg/L.

CONCLUSION

[] was assessed for acute toxicity to unfed *Daphnia magna* neonates, less than 24 hours old, in an unaerated, static, acute, 48-hour limit test. The 48-hour EC₅₀, based on the nominal limit test concentration of [] and immobility, was greater than 120 mg/L.

RECORDS AND SAMPLE STORAGE

Specimens (if applicable), raw data, the protocol, amendments (if any), and the final report will be retained at DuPont Haskell, Newark, Delaware, or at Iron Mountain Records Management, Wilmington, Delaware.

REFERENCES

1. Organisation for Economic Co-Operation and Development (OECD). Guideline for the Testing of Chemicals: 202, 13 April 2004.

Attachment 90 cont'd

[] Static, Acute,
48-Hour Limit Test with *Daphnia magna*

Revision 1
DuPont-[]

Table 2
Water Chemistry of the Dilution Water Control and Limit Test Concentration at Test Start

Nominal [] Concentration (mg/L)	Total Alkalinity (mg/L as CaCO ₃)	EDTA Hardness (mg/L as CaCO ₃)	Conductivity (μmhos/cm)
Dilution Water Control	51	131	255
120	52	127	285

Table 3
Dissolved Oxygen Concentration (mg/L)^Ψ of [] Test Solutions

Nominal [] Concentration (mg/L)	0 Hours		48 Hours	
	A [†]	C [†]	A [†]	C [†]
Dilution Water Control	8.5	8.5	8.7	8.7
120	8.5	8.5	8.8	8.7

Ψ The theoretical dissolved oxygen concentration at 100% saturation is 9.1 mg/L at 20°C.

† Replicate test chambers contained 5 daphnids each (total 30 daphnids for the limit test concentration and 20 for the dilution water control) at test start.

Attachment 90 cont'd

[] Static, Acute,
48-Hour Limit Test with *Daphnia magna*

Revision 1
DuPont-[]

Table 4
pH of [] Test Solutions

Nominal [] Concentration (mg/L)	0 Hours		48 Hours	
	A [†]	C [†]	A [†]	C [†]
Dilution Water Control	7.4	7.4	8.0	7.9
120	7.6	7.6	8.0	8.0

† Replicate test chambers contained 5 daphnids each (total 30 daphnids for the limit test concentration and 20 for the dilution water control) at test start.

Table 5
Temperature (°C) of [] Test Solutions

Nominal [] Concentration (mg/L)	0 Hours		48 Hours	
	A [†]	C [†]	A [†]	C [†]
Dilution Water Control	20.5	20.5	20.2	20.2
120	20.5	20.7	20.1	20.2

† Replicate test chambers contained 5 daphnids each (total 30 daphnids for the limit test concentration and 20 for the dilution water control) at test start.

Attachment 90 cont'd

[]: Static, Acute,
48-Hour Limit Test with *Daphnia magna*

Revision 1
DuPont-[]

Table 6

Immobility of *Daphnia magna* at 24 and 48 Hours in an Unaerated, Static, Acute, 48-Hour Limit Test with []

Nominal [] Concentration (mg/L)	Number Immobile / Number at Test Start											
	24 Hours						48 Hours					
	A [†]	B [†]	C [†]	D [†]	E [†]	F [†]	A [†]	B [†]	C [†]	D [†]	E [†]	F [†]
Dilution Water Control 120	0/5 0/5	0/5 0/5	0/5 0/5	0/5 0/5	- 0/5	- 0/5	0/5 0/5	0/5 0/5	0/5 0/5	0/5 0/5	- 0/5	- 0/5

† Replicate test chambers contained 5 daphnids each (total 30 daphnids for the limit test concentration and 20 for the dilution water control) at test start.

- Not applicable

Attachment 90 cont'd

[] Static, Acute,
48-Hour Limit Test with *Daphnia magna*

Revision 1
DuPont-[]

Table 7

Sublethal Effects in *Daphnia magna* at 24 and 48 Hours in an Unaerated, Static, Acute 48-Hour Limit Test with []

Nominal [] Concentration (mg/L)	Number Affected / Number Alive											
	24 Hours						48 Hours					
	A [†]	B [†]	C [†]	D [†]	E [†]	F [†]	A [†]	B [†]	C [†]	D [†]	E [†]	F [†]
Dilution Water Control 120	0/5 0/5	1 ^a /5 0/5	0/5 0/5	0/5 0/5	- 0/5	- 0/5	0/5 0/5	0/5 0/5	0/5 0/5	0/5 0/5	- 0/5	- 0/5

† Replicate test chambers contained 5 daphnids each (total 30 daphnids for the limit test concentration and 20 for the dilution water control) at test start.

- Not applicable

OBSERVATION KEY

a Daphnid floating at surface

Attachment 91 cont'd

[] Static, 72-Hour Growth Inhibition
Limit Test with the Green Alga, *Pseudokirchneriella subcapitata*

Revision 1
DuPont-[]

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study was conducted in compliance with U.S. EPA TSCA (40 CFR part 792) Good Laboratory Practice Standards, which are compatible with current OECD and MAFF (Japan) Good Laboratory Practices.

Study Director: [] — 11 JUL 2008
Date

Attachment 91 cont'd

[] Static, 72-Hour Growth Inhibition
Limit Test with the Green Alga, *Pseudokirchneriella subcapitata*

Revision 1
DuPont-[]

QUALITY ASSURANCE STATEMENT

Work Request Number: []
Service Code Number: []

Key inspections for DuPont work request [], service code [] were performed for the tasks completed at DuPont by the Quality Assurance Unit of DuPont and the findings were submitted on the following dates.

<i>Phase Audited</i>	<i>Audit Dates</i>	<i>Date Reported to Study Director</i>	<i>Date Reported to Management</i>
Protocol:	June 1, 2007	June 1, 2007	June 1, 2007
Conduct:	June 5, 2007	June 5, 2007	June 5, 2007
Report/Records:	December 4 & 6, 2007	December 7, 2007	December 10, 2007
Report Revision 1:	July 8, 2008	July 8, 2008	July 8, 2008

Reported by: []

11 July 2008
Date

Attachment 91 cont'd

[] Static, 72-Hour Growth Inhibition
Limit Test with the Green Alga, *Pseudokirchneriella subcapitata*

Revision 1
DuPont-[]

CERTIFICATION

We, the undersigned, declare that this report provides an accurate evaluation of data obtained from this study.

Reviewed by: [] 9 July 2008
Date

Issued by Study Director: [] 11 JUL 2008
Date

TABLE OF CONTENTS

	Page
GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT	2
QUALITY ASSURANCE STATEMENT	3
CERTIFICATION.....	4
LIST OF TABLES	6
LIST OF FIGURES	6
LIST OF APPENDICES	6
STUDY INFORMATION	7
REASON FOR REVISION 1.....	8
SUMMARY	8
INTRODUCTION.....	10
MATERIALS AND METHODS	10
A. Test Guidelines	10
B. Test Substance	10
C. Test Solution Preparation.....	10
D. AAP Nutrient Medium Preparation	10
E. Organism Culture.....	10
F. Study Methods	11
G. Sample Preparation and Chemical Analysis.....	12
H. Statistical Analyses	12
RESULTS AND DISCUSSION	15
A. Analytical Report.....	15
B. In-Life Report	15
C. Statistical Report.....	16
CONCLUSIONS	16
RECORDS AND SAMPLE STORAGE	16
REFERENCES.....	16
TABLES.....	18
FIGURE.....	25
APPENDICES.....	27

REASON FOR REVISION 1

To provide consistent reporting of endpoints among studies based on guidance contained in OECD TG 201, 202 and 203.

SUMMARY

The toxicity of [] to the green algae, *Pseudokirchneriella subcapitata*, was determined in a 72-hour, static limit test. The test was conducted in accordance with OECD Guideline for the Testing of Chemicals: 201 (2006).

The purity of [] was [] by analysis. The study was conducted with a blank control and a nominal limit test concentration of 120 mg/L [] (106 mg/L mean, measured) at a mean lighting intensity of 5890 lux (range of 5650 to 6080 lux), a mean temperature of 23.9°C (range of 23.8 to 24.0°C), and a shaking speed of 95 rpm. The mean, measured limit test concentration was 80-120% of the nominal limit test concentration for the study. Synthetic algal-assay-procedure (AAP) nutrient medium was used as the test diluent and blank control. Test solutions were not renewed. Six replicates were used for the limit test concentration and the blank control. A single test flask was used for the abiotic (stability) control. Healthy cell count, area under the growth curve, and growth rate were determined at 24-hour intervals over the 72-hour test.

Inhibition of cell growth expressed as biomass (cell number), area under the growth curve, and average specific growth rate of *Pseudokirchneriella subcapitata* exposed to a nominal limit test concentration of 120 mg/L [] for 72 hours was -2, -4, and 0%, respectively.^a Healthy cell counts increased in the blank control by at least a factor of 16 in 72 hours, the coefficient of variation of average specific growth rates during the whole test period (0-72 hours) in blank control replicates did not exceed 7%, and the mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2, and 2-3) in the blank control replicates did not exceed 35%, thereby satisfying the appropriate test acceptance criteria. The nominal [] concentrations in the limit test concentration and abiotic control were each 120 mg/L. The Day 0 measured concentrations were 105 and 105 mg/L, and the 72-hour measured concentrations were 107 and 109 mg/L, respectively.

No significant inhibition was seen at the nominal limit test concentration of 120 mg/L []. The E_bC_{50} (0-72 hour) values, based on the nominal limit test concentration and cell count and area under the growth curve were both greater than 120 mg/L. The E_rC_{50} (0-72 hour) value, based on the nominal limit test concentration and growth rate was greater than 120 mg/L. The LOEC values, based on the nominal limit test concentration and cell count, area under the growth curve, and growth rate, were each greater than 120 mg/L []. The NOEC values,

^a Negative values indicate stimulation of growth.

Attachment 91 cont'd

[] Static, 72-Hour Growth Inhibition
Limit Test with the Green Alga, *Pseudokirchneriella subcapitata*

Revision 1
DuPont-[]

based on the nominal limit test concentration and cell count, area under the growth curve, and growth rate, were each 120 mg/L [].^a

The results are summarized as follows:

Nominal concentrations of [], mg/L	Blank control, 120 (limit test concentration), and abiotic control (120)
Day 0 measured concentrations of [], mg/L	ND,* 105, and 105
72-hour measured concentrations of [], mg/L	ND,* 107, and 109
Mean, measured concentrations of [], mg/L	ND,* 106 (limit test concentration), and 107 (abiotic control)
E _b C ₅₀ (0-72 hour) for [], based on nominal concentration, mg/L	Cell Count: greater than 120
	Area Under Curve: greater than 120
E _r C ₅₀ (0-72 hour) for [], based on nominal concentration, mg/L	Growth Rate: greater than 120
72-hour LOEC for [], based on nominal concentration, mg/L	Cell Count: greater than 120
	Area Under Curve: greater than 120
	Growth Rate: greater than 120
72-hour NOEC for [], based on nominal concentration, mg/L	Cell Count: 120
	Area Under Curve: 120
	Growth Rate: 120

* ND denotes not detected. The limit of detection for [] was calculated as 0.0001 µg/L for day 0 and day 3.

^a The E_bC₅₀ (0-72 hour) is defined as the "effective concentration" producing a 50% inhibition of growth based on the 72-hour cell count (density) or area under the growth curve relative to the control. The E_rC₅₀ (0-72 hour) is defined as the "effective concentration" producing a 50% inhibition of growth based on the 0-72 hour growth rate relative to the control. The LOEC is defined as the lowest concentration of test substance that had a significant effect on the measured parameter relative to the control. The NOEC is defined as the highest concentration of test substance that had no significant effect on the measured parameter relative to the control.

RESULTS AND DISCUSSION

A. Analytical Report

A full description of the results, including representative chromatograms, is presented in Appendix B. The measured concentrations of [] in the day 0 limit test concentration and abiotic control solutions were [] of the targeted nominal test concentrations adjusted for test substance purity of []. The measured concentrations of [] in the day 3 test solutions were [], respectively, of the targeted nominal test concentrations adjusted for test substance purity of []. The mean, measured concentrations of [] in the test solutions were [] respectively, of the targeted nominal test concentrations adjusted for test substance purity of []. The blank control solutions contained no detectable concentrations of [] on either day 0 or day 3.

B. In-Life Report

The nominal limit test concentration for the definitive test was 120 mg/L []. The nominal concentration of the abiotic control was 120 mg/L []. A culture medium blank control was used in this study. The nominal [] concentrations ([] active ingredient) in the 120 mg/L and abiotic control solutions were each 99.1 mg/L. The corresponding mean, measured concentrations were 106 and 107 mg/L. The mean, measured concentrations were within 80-120% of the nominal limit test concentration for the study.

All environmental parameters for the definitive test (Tables 1 and 2) were within expected ranges. During the test, the shaking speed was 95 rpm, pH ranged from 7.01 to 7.83, mean lighting was 5890 lux with a range of 5650 to 6080 lux, and temperature in the environmental chamber ranged from 23.8 to 24.0°C.

Data on healthy cell count, area under the growth curve, and growth rate are presented in Tables 3, 4, and 5, respectively. Growth curves for the blank control solution and test solution are presented in Figure 1. Healthy cell counts increased in the blank control by a factor of approximately 240 in 72 hours, the mean coefficient of variation for section-by-section specific growth rates in the blank control was 17.71%, and the coefficient of variation of the average specific growth rate during the 72-hour exposure period in the blank control replicates was 1.87%, thereby satisfying the appropriate test acceptance criteria.

Inhibition of growth based on healthy cell count, area under the growth curve, and growth rate of *P. subcapitata* exposed to a nominal limit test concentration of 120 mg/L [] for 72 hours was -2, -4, and 0%, respectively. A summary of the 72-hour EC₅₀, LOEC, and NOEC values is presented in Table 6. The E_bC₅₀ (0-72 hour) and E_rC₅₀ (0-72 hour) values, based on the nominal concentration and cell count, area under the growth curve, and growth rate, were each greater than 120 mg/L. The 72-hour LOEC values, based on the nominal concentration and cell count, area under the growth curve, and growth rate, were each greater than 120 mg/L. The 72-hour NOEC values, based on the nominal concentration and healthy cell count, area under the growth curve, or growth rate, were each 120 mg/L.

C. Statistical Report

The data for healthy cell count, and growth rate based on healthy cell count were determined to be normally distributed (Shapiro-Wilk test⁽⁵⁾) with equal variances (Levene's test⁽⁶⁾). Therefore, the Jonckheere-Terpstra⁽⁸⁾ trend test was used to determine the LOEC and NOEC values. The data for area under the growth curve were determined to be non-normally distributed (Shapiro-Wilk test⁽⁵⁾). Therefore, a non-parametric analysis was performed (Kruskal-Wallis test⁽⁸⁾) and the Jonckheere-Terpstra test⁽⁸⁾ was used to determine the LOEC and NOEC values. No outliers were found⁽¹¹⁾ in the data for healthy cell count, area under the growth curve, and growth rate based on healthy cell count. The LOEC and NOEC values for healthy cell count, area under the growth curve, and growth rate based on healthy cell count were determined to be >120 mg/L and 120 mg/L, respectively.

The MAXSD test⁽¹²⁾ was used to test the null hypothesis that growth inhibition was greater than or equal to 50%. The growth inhibition at the limit test concentration was also not found to be statistically significantly different from the blank control, and therefore the E_bC_{50} and E_rC_{50} values for healthy cell count, area under the growth curve, and growth rate based on healthy cell count were determined to be greater than 120 mg/L.⁽³⁾

CONCLUSIONS

[] was assessed for toxicity to *Pseudokirchneriella subcapitata* in a static 72-hour test. There was no significant inhibitory effect on the growth and reproduction of *Pseudokirchneriella subcapitata* when exposed to a nominal concentration of 120 mg/L [] for 72 hours. Therefore, the E_bC_{50} (0-72 hour) and E_rC_{50} (0-72 hour) values, based on the nominal concentration and cell count, area under the growth curve, or growth rate, are greater than 120 mg/L []. The 72-hour LOEC values, based on the nominal concentration and cell count, area under the growth curve, and growth rate, are each greater than 120 mg/L []. The 72-hour NOEC values, based on the nominal concentration and healthy cell count, area under the growth curve, or growth rate, are each 120 mg/L.

RECORDS AND SAMPLE STORAGE

Specimens (if applicable), raw data, the protocol, amendments (if any), and the final report will be retained at Haskell Laboratory, Newark, Delaware, or at Iron Mountain Records Management, Wilmington, Delaware.

REFERENCES

1. Miller, W.E., Greene, J.C., and Shiroyama, T. (1978). The *Selenastrum capricornutum* Printz Algal Assay Bottle Test, EPA-600/9-78-018. U.S. Government Printing Office, Washington, DC.

Attachment 91 cont'd

[] Static, 72-Hour Growth Inhibition
Limit Test with the Green Alga, *Pseudokirchneriella subcapitata*

Revision 1
DuPont-[]

Table 1
pH Measurements of Test Solutions

Nominal [] Concentration	pH	
	0-Hour (Day 0)	72-Hour (Day 3)
Blank Control	7.61	7.83
120 mg/L	7.30	7.01
Abiotic Control: 120 mg/L	7.30	7.25

Table 2
Test Conditions: Chamber Light Intensity, Shaking Speed, and Temperature Range

Mean Light Intensity at Test Initiation ^a (lux)	Light Intensity Range at Test Initiation (lux)	Oscillations (rpm)	Temperature (°C)
5890	5650 to 6080	95	23.8 to 24.0

a mean of 3 measurements

Attachment 91 cont'd

[] Static, 72-Hour Growth Inhibition
Limit Test with the Green Alga, *Pseudokirchneriella subcapitata*

Revision 1
DuPont-[]

Table 3
Healthy Cell Count Data Summary

Nominal [Concentration mg/L	Rep.	Exposure Initiated: Day 0: 05 June 2007		Exposure Ended: Day 3: 08 June 2007	
		Healthy Cells/mL Count by Test Day			
		0	1	2	3
Blank Control	1	10,000	90,000	400,000	2,170,000
	2	10,000	60,000	410,000	2,610,000
	3	10,000	70,000	380,000	2,630,000
	4	10,000	40,000	490,000	2,390,000
	5	10,000	40,000	570,000	2,310,000
	6	10,000	60,000	340,000	2,030,000
Mean		10,000	60,000	431,667	2,356,667
Std. Dev.		0	18,974	83,766	238,216
Coeff. of Variation		0.0	31.6	19.4	10.1
120	1	10,000	80,000	300,000	2,270,000
	2	10,000	30,000	270,000	2,350,000
	3	10,000	40,000	650,000	2,240,000
	4	10,000	50,000	490,000	2,550,000
	5	10,000	50,000	570,000	2,520,000
	6	10,000	60,000	590,000	2,480,000
Mean		10,000	51,667	478,333	2,401,667
Std. Dev.		0	17,224	158,545	132,878
Coeff. of Variation		0.0	33.3	33.1	5.5
% Inhibition		0	14	-11	-2

Attachment 91 cont'd

[] Static, 72-Hour Growth Inhibition
Limit Test with the Green Alga, *Pseudokirchneriella subcapitata*

Revision 1
DuPont-[]

Table 4
Area Under the Growth Curve Data Summary

Nominal [Concentration mg/L	Rep.	Exposure Initiated: Day 0: 05 June 2007		Exposure Ended: Day 3: 08 June 2007	
		Area Under the Growth Curve Based on Healthy Cells/mL Count by Test Day			
		Day 0-1	Day 0-2	Day 0-3	
Blank Control	1	40,000	275,000	1,550,000	
	2	25,000	250,000	1,750,000	
	3	30,000	245,000	1,740,000	
	4	15,000	270,000	1,700,000	
	5	15,000	310,000	1,740,000	
	6	25,000	215,000	1,390,000	
Mean		25,000	260,833	1,645,000	
Std. Dev.		9,487	32,158	145,705	
Coeff. of Variation		37.9	12.3	8.9	
120	1	35,000	215,000	1,490,000	
	2	10,000	150,000	1,450,000	
	3	15,000	350,000	1,785,000	
	4	20,000	280,000	1,790,000	
	5	20,000	320,000	1,855,000	
	6	25,000	340,000	1,865,000	
Mean		20,833	275,833	1,705,833	
Std. Dev.		8,612	78,893	185,995	
Coeff. of Variation		41.3	28.6	10.9	
% Inhibition		17	-6	-4	

Attachment 91 cont'd

[] Static, 72-Hour Growth Inhibition
Limit Test with the Green Alga, *Pseudokirchneriella subcapitata*

Revision 1
DuPont-[]

Table 5
Growth Rate Data Summary

Nominal [Concentration] mg/L	Rep.	Exposure Initiated: Day 0: 05 June 2007		Exposure Ended: Day 3: 08 June 2007	
		Growth Rate Based on Healthy Cells/mL Count by Test Day			
		Day 0-1	Day 0-2	Day 0-3	
Blank Control	1	2.20	1.84	1.79	
	2	1.79	1.86	1.85	
	3	1.95	1.82	1.86	
	4	1.39	1.95	1.83	
	5	1.39	2.02	1.81	
	6	1.79	1.76	1.77	
Mean		1.75	1.88	1.82	
Std. Dev.		0.32	0.09	0.03	
Coeff. of Variation		18.19	4.97	1.87	
120	1	2.08	1.70	1.81	
	2	1.10	1.65	1.82	
	3	1.39	2.09	1.80	
	4	1.61	1.95	1.85	
	5	1.61	2.02	1.84	
	6	1.79	2.04	1.84	
Mean		1.60	1.91	1.83	
Std. Dev.		0.34	0.19	0.02	
Coeff. of Variation		21.04	9.79	1.01	
% Inhibition		9	-2	0	

Attachment 91 cont'd

[] Static, 72-Hour Growth Inhibition
Limit Test with the Green Alga, *Pseudokirchneriella subcapitata*

Revision 1
DuPont-[]

Table 5
Growth Rate Data Summary (continued)

Nominal [Concentration mg/L	Rep.	Exposure Initiated:		Exposure Ended:			
		Day 0: 05 June 2007		Day 3: 08 June 2007			
		Growth Rate Based on Healthy Cells/mL Count by Test Day					
		Day 0-1	Day 1-2	Day 2-3	Mean	Std. Dev.	Co of Var
Blank Control	1	2.20	1.49	1.69	1.79	0.36	20.28
	2	1.79	1.92	1.85	1.85	0.07	3.51
	3	1.95	1.69	1.93	1.86	0.14	7.73
	4	1.39	2.51	1.58	1.83	0.60	32.72
	5	1.39	2.66	1.40	1.81	0.73	40.23
	6	1.79	1.73	1.79	1.77	0.03	1.79
Mean		1.75	2.00	1.71	1.82		17.71
Std. Dev.		0.32	0.47	0.19	0.03		
Coeff. of Variation		18.19	23.63	11.38	1.87		
120	1	2.08	1.32	2.02	1.81	0.42	23.35
	2	1.10	2.20	2.16	1.82	0.62	34.33
	3	1.39	2.79	1.24	1.80	0.86	47.43
	4	1.61	2.28	1.65	1.85	0.38	20.44
	5	1.61	2.43	1.49	1.84	0.52	27.94
	6	1.79	2.29	1.44	1.84	0.43	23.22
Mean		1.60	2.22	1.67			29.45
Std. Dev.		0.34	0.49	0.36			
Coeff. of Variation		21.04	21.94	21.56			
% Inhibition		9	-11	2			

Attachment 91 cont'd

[] Static, 72-Hour Growth Inhibition
Limit Test with the Green Alga, *Pseudokirchneriella subcapitata*

Revision 1
DuPont-[]

Table 6
72-Hour EC₅₀, LOEC, and NOEC Values for *Pseudokirchneriella subcapitata* Based on the
Nominal Concentration of []

Parameter	72-Hour E _x C ₅₀ *	Model	72-Hour LOEC	72-Hour NOEC	Method
Healthy Cell Count	> 120 mg/L	MAXSD	> 120 mg/L	120 mg/L	t-test
Area Under the Growth Curve	> 120 mg/L	MAXSD	> 120 mg/L	120 mg/L	t-test
Growth Rate	> 120 mg/L	MAXSD	> 120 mg/L	120 mg/L	t-test

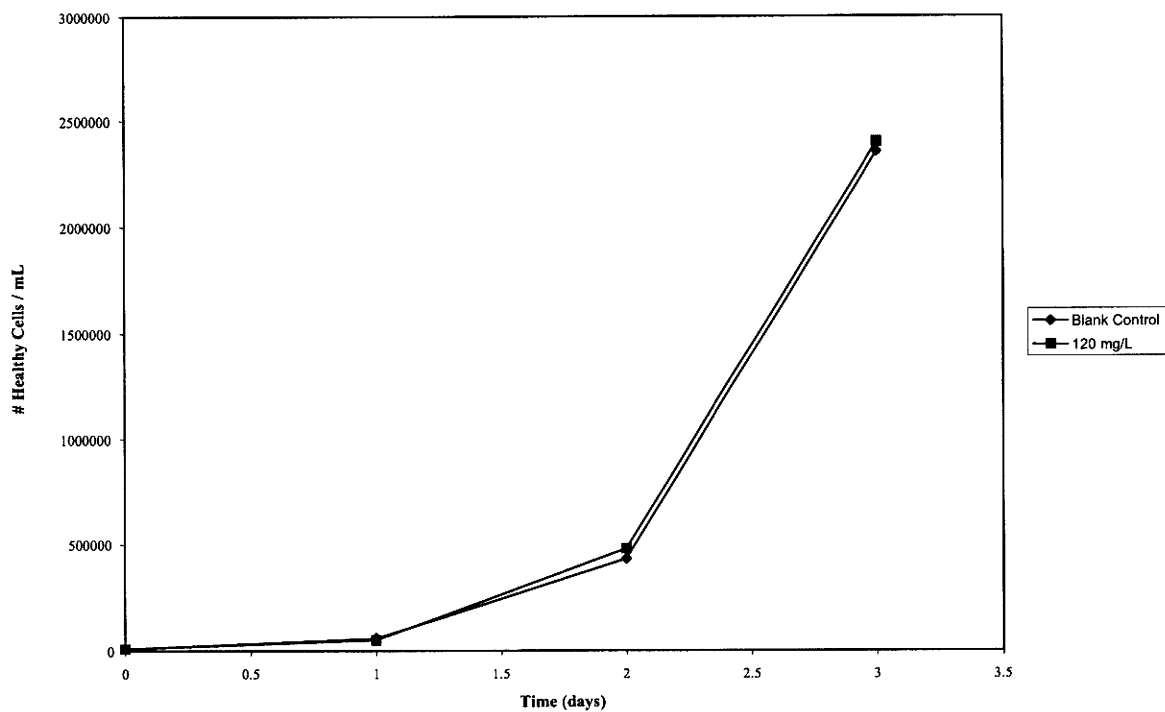
* x = b for healthy cell count and area under growth curve; r for growth rate.

Attachment 91 cont'd

[] Static, 72-Hour Growth Inhibition
Limit Test with the Green Alga, *Pseudokirchneriella subcapitata*

Revision 1
DuPont-[]

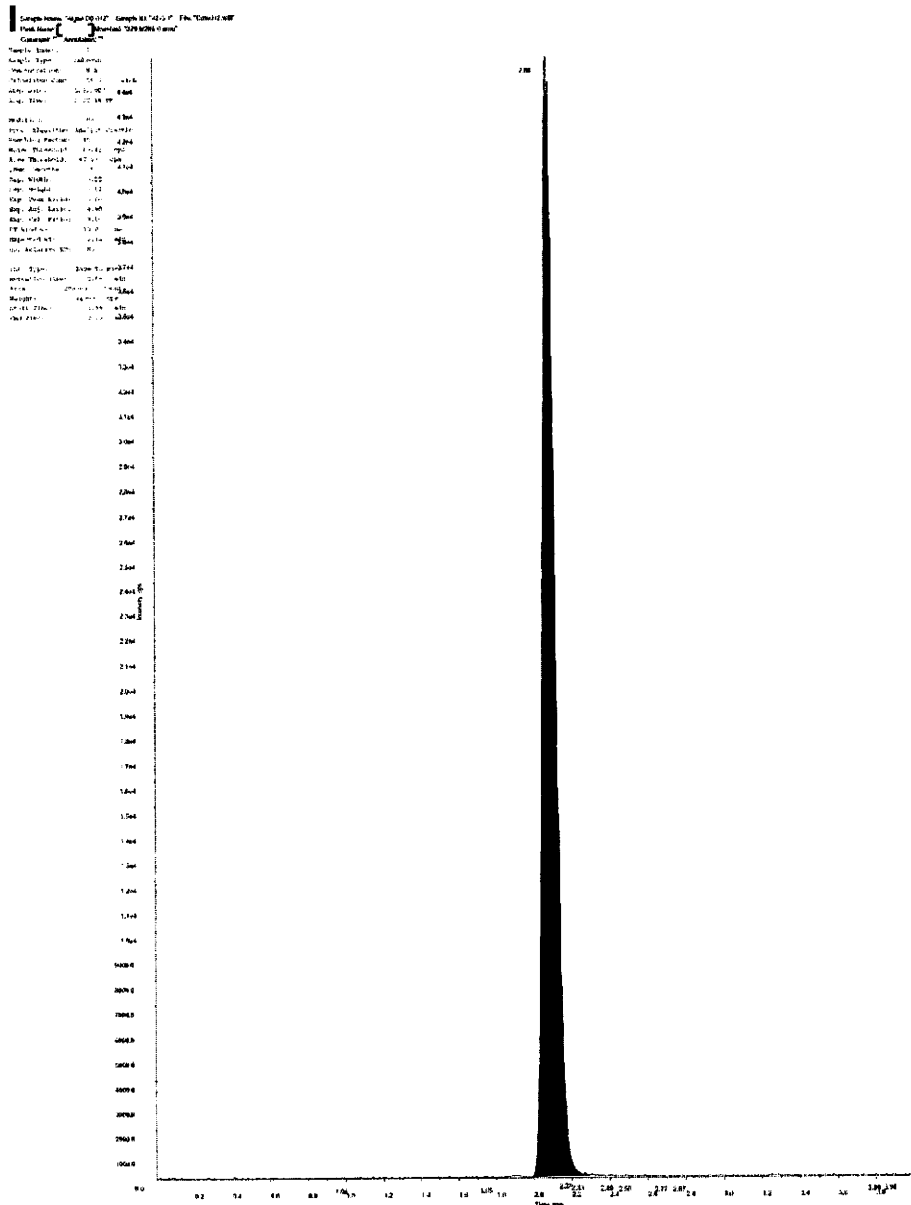
Figure 1
Healthy Cell Count Versus Time for *Pseudokirchneriella subcapitata* Based on the Nominal
Concentration of []



DuPont []

FIGURE 4

REPRESENTATIVE CHROMATOGRAM OF A [] TEST SOLUTION



[] elutes at a retention time of approximately 2.08 minutes. The test solution sample contained [] at a nominal concentration of 60 µg/L. The sample was diluted 2000x with acetonitrile/water, 50/50, v/v, prior to analysis.